JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.009025

Effect of Dietary *Curcuma, Capsicum,* and *Lentinus* on Enhancing Local Immunity against *Eimeria acervulina* Infection

Sung Hyen Lee[1], Hyun S. Lillehoj[1], Seung Ik. Jang[1], Duk Kyung Kim[1], Catherine Ionescu[2] and David Bravo[2]

[1] Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service-U.S. Department of Agriculture, Beltsville, MD, USA.
[2] Pancosma S.A., Voie-des-Traz 6, CH-1218 Le Grand Saconnex, Geneva, Switzerland

The protective effect of orally administered *Curcuma longa* (turmeric), *Capsicum annuum* and C. *frutescens* (hot pepper), and *Lentinus edodes* (shiitake mushroom) on avian coccidiosis was evaluated in young broilers. Broiler chickens were continuously fed with a standard diet or standard diet supplemented with *Curcuma, Capsicum/Lentinus* or *Curcuma/Capsicum/Lentinus* from hatch and body weight gains, fecal oocyst shedding, antibody titers, and pro-inflammatory cytokine gene expression were measured as parameters of protective immunity following challenge infection with *E. acervulina.* Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet showed significantly improved body weight gains compared with birds on the standard diet or birds given *Capsicum/Lentinus*-supplemented diet following challenge infection with *E. acervulina.* Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet shed significantly reduced fecal oocysts and produced higher serum antibody titers compared with the groups fed the standard diet or fed *Curcuma* or *Capsicum/Lentinus.* Finally, the levels of local cytokine transcripts of IL-1β, IL-6, IL-15, and IFN-γ were consistently greater in the *Curcuma/Capsicum/Lentinus*-fed group compared to the controls fed only the standard diet, *Curcuma,* or *Capsicum/Lentinus* groups. This study provides first immunological evidence that dietary supplementation of turmeric, hot pepper, and shiitake mixture significantly enhances local innate immunity and provides higher protective immunity against *E. acervulina* infection.

Key words: coccidiosis, cytokines, hot pepper, immunity, shiitake, turmeric

J. Poult. Sci., 47: 89–95, 2010

Introduction

Avian coccidiosis is an intestinal disease caused by several species of *Eimeria* protozoa and represents an economically important parasitic infection for the poultry industry worldwide (Lillehoj and Lillehoj, 2000). Due to increasing regulations with the use of prophylactic drugs, high cost of vaccines, and escalating consumers' interest on naturally-raised chickens, much interest has been devoted toward the development of alternative strategies to control avian coccidiosis (Lillehoj and Lee, 2007a, b). It is now well accepted that many medicinal food and herbal products are highly effective promoting host defense mechanisms against microbial infections, tumors, and oxidative stress (Park *et al.,* 2004; Lee *et al.,* 2005, 2007a, d, 2008b). For example, enhanced resistance against many infectious diseases including coccidiosis was demonstrated using dietary feeding of plant-derived phytonutrients (Banfield *et al.,* 2002; Lee *et al.,* 2007a, 2008c; Naidoo *et al.,* 2008).

Hot pepper (*Capsicum* spp.) is a vegetable of importance in human nutrition and has many beneficial effects on human health. For example, *C. frutescens* is desired for its pungency, pigments, and its physiological and pharmaceutical uses, *C. annuum* exerts anti-oxidative effects *in vitro* (Conforti *et al.,* 2007) and prevents Fe^{2+}-induced lipid peroxidation in brain (Oboh *et al.,* 2007) and *Capsicum* oleoresin, prepared by organic extraction of pepper fruits, contains anti-bacterial activity and is effective in treating stomach illnesses (Spices board, 2008). Plants of the genus *Curcuma,* including *C. longa* (turmeric), have anti-oxidant and anti-inflammatory properties, and *C. amada* and *C. caesi* inhibit the growth of gram positive and negative bacteria as well as *Candida albicans* (Policegoudra *et al.,* 2007; Sodsai *et al.,* 2007; Mannangatti and Narayanasamy, 2008). *Lentinus edodes* (shiitake mush-

Received: April 3, 2009, Accepted: August 6, 2009
Released Online Advance Publication: December 10, 2009
Correspondence: H.S. Lillehoj, Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service-United States Department of Agriculture, Building 1043, BARC-East, Beltsville, MD 20705. (E-mail: Hyun.Lillehoj@ars.usda.gov)
‡ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

room) with its well known medicinal benefits, especially anti-tumor and anti-viral properties, have been effective in treating atopic diseases and arthritis (Park *et al.,* 2004).

These broad spectrums of biological properties suggest that these plant foods contain multiple beneficial phytonutrients operating through a variety of different mechanisms, and led us to hypothesize that they may protect against infectious diseases in poultry. Therefore, to evaluate their effects in enhancing innate immunity to avian coccidiosis, we compared seven parameters of protective immunity against coccidiosis (weight gain, fecal oocyst shedding, *Eimeria* antibody titers, and expression of IL-1β, IL-6, IL-15, and IFN-γ) in broiler chickens fed a diet containing *Curcuma* and/or *Capsicum/Lentinus.*

Materials and Methods

Experimental Animals

All experiments were approved by the Beltsville Agriculture Research Center-Small Animal Care and Use Committee. One day-old broiler chickens (Ross/Ross, Longenecker's Hatchery, Elizabethtown, PA) were housed in Petersime starter brooder units and randomly assigned to 5 groups (20 birds/group). The birds were kept in brooder pens in an *Eimeria*-free facility for 2 weeks and transferred into large hanging cages (2 birds/cage) in a separate location where they were infected and kept until the end of experimental period.

Experimental Diets

All food extracts were obtained from Pancosma S.A. (Geneva, Switzerland). Crushed *Capsicum annuum* and *C. frutescens* were extracted with volatile solvents leading to an oleoresin and finally processed to produce a powder. And aqueous extract of *Lentinus edodes* was prepared at 95℃ with pressure and spray-drying. Based on our preliminary results, *Capsicum* extract was always used in combination with *Lentinus* extract since this combination provided greater protection against experimental coccidiosis compared with either extract alone (unpublished data). *Curcuma longa* rhizomes were extracted with organic solvents. Chickens were continuously fed from hatch with a standard diet alone (control) or with diets supplemented with 35 mg/kg of *C. longa* alone (diet M), 35 mg/kg of *C. annuum* and *C. frutescens* plus 5 mg/kg of *L. edodes* (diet S), or *Curcuma, Capsicum,* and *Lentinus* at these concentrations (diet SM).

Eimeria Infection and Body Weight and Fecal Oocysts Measurements

Body weights of chickens were measured at days 1, 3, 14, 19, and 24 post-hatch to evaluate the effects of various diets on body weight changes. At day 14 post-hatch, chickens were either uninfected or orally infected with 2.0 $\times 10^4$ sporulated oocysts of *E. acervulina* as described by Lee *et al.* (2008a). Body weight gains were calculated over the period between days 0 and 10 post-infection. For determination of fecal oocyst shedding, birds were placed in oocyst collection cages (2 birds/cage) and fecal samples were collected from day 5 to day 10 post-infection. Oocyst numbers were determined as described (Lee *et al.,* 2007a, b, 2008d) using a McMaster chamber according to the formula: Total oocysts/bird = oocyst count × dilution factor × (fecal sample volume/counting chamber volume)/2.

Serum Antibody Levels

Serum samples were obtained at day 10 post-infection (4 birds/group) and tested by ELISA to determine serum antibody levels against an apicomplexa antigen, EtMIC2, as described (Lee *et al.* 2007b, c). Briefly, 96-well microtiter plates were coated overnight with 10 μg/well of purified recombinant EtMIC2 protein. The plates were washed with PBS containing 0.05% Tween (PBS-T), and blocked with PBS containing 1% BSA. Diluted sera (1: 100) were added (100 μL/well), incubated with agitation for 1 hr at room temperature, washed with PBS-T, and bound antibody detected with peroxidase-conjugated rabbit anti-chicken IgG (Sigma, St. Louis, MO) and peroxidase-reactive substrates made of phosphate citrate buffer (Sigma, St. Louis, MO), 3,3′,5,5′-Tetramethylbenzidine (Sigma, St. Louis, MO), and hydrogen peroxide (Sigma, St. Louis, MO). Optical density at 450 nm (OD_{450}) was determined with a microplate reader (Bio-Rad, Richmond, CA) and all experimental samples were analyzed in triplicates.

Quantification of Cytokine mRNA Levels

Intestinal duodenum tissues were obtained from uninfected chickens which were fed with various diets supplemented with plant extracts at day 14 post-hatch (4 birds/group), and local cytokine gene expression was determined using real time RT-PCR as previously described (Hong *et al.,* 2006a, b). The intestinal duodenum was removed, cut longitudinally, and washed three times with ice-cold Hanks' balanced salt solution (HBSS) containing 100 U/mL of penicillin and 100 μg/mL of streptomycin. The mucosal layer was carefully scraped away using a surgical scalpel and the tissue was washed with HBSS. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA). Five micrograms of total RNA were treated with 1.0 U of DNase I and 1.0 μL of 10X reaction buffer (Sigma), incubated for 15 min at room temperature, 1.0 μL of stop solution was added to inactivate DNase I, and the mixture was heated at 70℃ for 10 min. RNA was reverse-transcribed using the StrataScript first-strand synthesis system (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. Briefly, 5.0 μg of RNA was combined with 10X the first strand buffer, 1.0 μL of oligo (dT) primer (5.0 μg/μL), 0.8 μL of dNTP mix (25 mM of each dNTP), and RNase-free water to a total volume of 19 μL. The mixture was incubated at 65℃ for 5 min, cooled to room temperature, 50 U of StrataScript reverse transcriptase was added, the mixture was incubated at 42℃ for 1 hr, and the reaction was stopped by heating at 70℃ for 5 min. Quantitative RT-PCR oligonucleotide primers for chicken cytokines and the GAPDH internal control are listed in Table 1. Amplification and detection were carried out using equivalent amounts of total RNA using the Mx3000P system and

Brilliant SYBR Green qPCR master mix (Stratagene). Standard curves were generated using $\log_{10}$ diluted standard RNA. Levels of individual transcripts were then normalized to those of GAPDH analyzed by the Q-gene program (Muller *et al.,* 2002). Each analysis was performed in triplicates. To normalize individual replicates, the logarithmic-scaled threshold cycle (C_t) values were transformed to linear units of normalized expression prior to calculating means and SEM for the references and individual targets, followed by the determination of mean normalized expression (MNE) using the Q-gene program (Lee *et al.,* 2008a, c).

Statistical Analyses

Statistical analyses were performed using SPSS software (SPSS 15.0 K for Windows, Chicago, IL), and all data were expressed as means±SEM values. Comparisons of the mean values were performed by one-way analysis of variance, followed by the Duncan's multiple range test and differences were considered statistically significant at $P<0.05$.

Results

Body Weight Change

Dietary feeding of broiler chickens with diets containing the *Curcuma, Capsicum,* and/or *Lentinus* did not show any toxic effects on host based upon body weight changes and other physical appearance. Over the first 14 days post-hatch, birds given the supplemented diets did not exhibit any changes in body weights compared with those which were on standard diet alone (Table 2). Following *E. acervulina* infection, however, chickens fed the M or SM diet exhibited increased weight gains starting from day 0 to day 10 post-infection (days 14 and 24 post-hatch) compared with the infected controls given the non-supplemented diet (Fig. 1). Chickens fed the SM diet gained body weight by significantly greater extent than the birds given the S diet.

Oocyst Production

Fecal oocyst shedding was significantly reduced by 51% in chickens fed the SM diet (4.6×10^7) compared with the infected group given the non-supplemented diet (9.3×10^7). In contrast, neither the M nor the S diet alone

Table 1. **Oligonucleotide primers used for quantitative RT-PCR of chicken cytokines**

RNA target	Primer sequences	PCR product size (bp)	Accession no.
GAPDH			
Forward	5′-GGTGGTGCTAAGCGTGTTAT-3′	264	*K01458*
Reverse	5′-ACCTCTGTCATCTCTCCACA-3′		
IL-1β			
Forward	5′-TGGGCATCAAGGGCTACA-3′	244	*Y15006*
Reverse	5′-TCGGGTTGGTTGGTGATG-3′		
IL-6			
Forward	5′-CAAGGTGACGGAGGAGGAC-3′	254	*AJ309540*
Reverse	5′-TGGCGAGGAGGGATTTCT-3′		
IL-15			
Forward	5′-TCTGTTCTTCTGTTCTGAGTGATG-3′	243	*AF139097*
Reverse	5′-AGTGATTTGCTTCTGTCTTTGGTA-3′		
IFN-γ			
Forward	5′-AGCTGACGGTGGACCTATTATT-3′	259	*Y07922*
Reverse	5′-GGCTTTGCGCTGGATTC-3’		

Table 2. **Body weights of the birds fed plant-supplemented diets**

Groups/days post-hatch	1	3	14	19	24
Uninfected control	41.9 ± 0.8^{NS}	78.5 ± 2.6^{NS}	348.8 ± 13.4^{NS}	630.6 ± 17.3^{ab}	869.0 ± 19.6^{ab}
Infected control	41.4 ± 0.9	75.8 ± 2.9	341.3 ± 24.4	597.1 ± 43.2^{b}	835.6 ± 46.7^{b}
S	41.1 ± 1.2	76.0 ± 3.0	363.2 ± 15.2	628.3 ± 19.9^{ab}	863.6 ± 26.6^{ab}
M	44.0 ± 0.8	84.0 ± 2.9	390.9 ± 8.2	686.0 ± 16.9^{a}	943.1 ± 19.1^{a}
SM	41.3 ± 0.9	83.4 ± 2.2	380.8 ± 9.9	671.8 ± 16.0^{ab}	953.8 ± 22.2^{a}

One-day-old broiler chickens were fed a standard diet or a standard diet supplemented with *C. annuum* and *C. frutescens* plus *L. edodes* (S), *C. longa* (M), or *C. annuum* and *C. frutescens, L. edodes,* and *C. longa* (SM), and their body weights were measured at various days post-hatch. Values indicate means±SEM. Within each group, values not sharing the same letters are significantly different ($P<0.05$) according to the Duncan's multiple range test. NS=not significant.

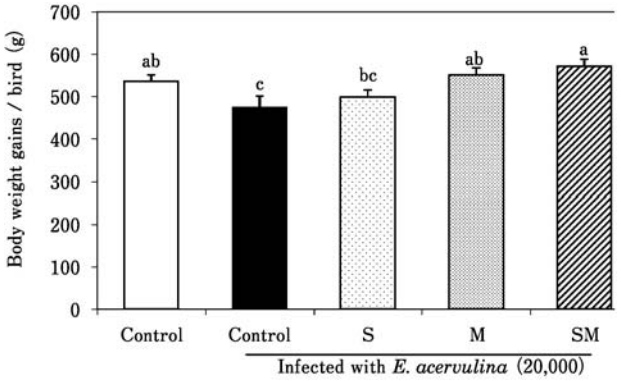

Fig. 1. **The effect of dietary plant extracts on body weight gains of birds infected with *E. acervulina*.** One-day-old broiler chickens were fed a standard diet alone (Control) or a standard diet supplemented with *C. annuum* and *C. frutescens* plus *L. edodes* (S), *C. longa* (M), or *C. annuum* and *C. frutescens, L. edodes,* and *C. longa* (SM). At 14 days post-hatch, the chickens were uninfected or infected with 2.0×10^4 sporulated oocysts of *E. acervulina,* and their body weight gains were measured starting from day 0 to day 10 post-infection. Each bar represents the mean±SEM values. Bars not sharing the same letters are significantly different ($P<0.05$), according to the Duncan's multiple range test.

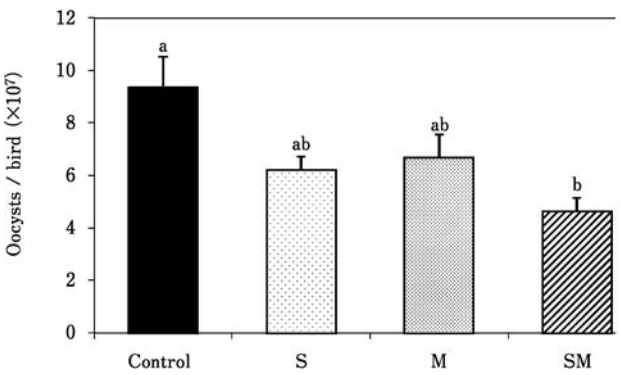

Fig. 2. **The effect of dietary plant extracts on fecal oocyst shedding from birds infected with *E. acervulina*.** One-day-old broiler chickens were fed a standard diet alone (Control) or a standard diet supplemented with *C. annuum* and *frutescens* plus *L. edodes* (S), *C. longa* (M), or *C. annuum* and *frutescens, L. edodes,* and *C. longa* (SM). At 14 days post-hatch, the chickens were infected with 2.0×10^4 sporulated oocysts of *E. acervulina* and fecal oocysts were enumerated starting from day 5 to day 10 post-infection. Each bar represents the mean±SEM values. Bars not sharing the same letters are significantly different ($P<0.05$) according to the Duncan's multiple range test.

reduced oocyst shedding compared to the control standard diet (Figure 2). No oocysts were detected from the uninfected control chickens (data not shown).

Serum Antibody Responses

The levels of serum antibodies reactive with the EtMIC 2, an apicomplexa protein, were increased in *E. acervulina*-infected chickens fed the SM diet (0.34±0.02), but not in birds given extracts of M diet (0.26±0.01) or S diet (0.23 ±0.04), when compared to the control birds on standard diet (0.21±0.02) following *E. acervulina* infection (Figure 3).

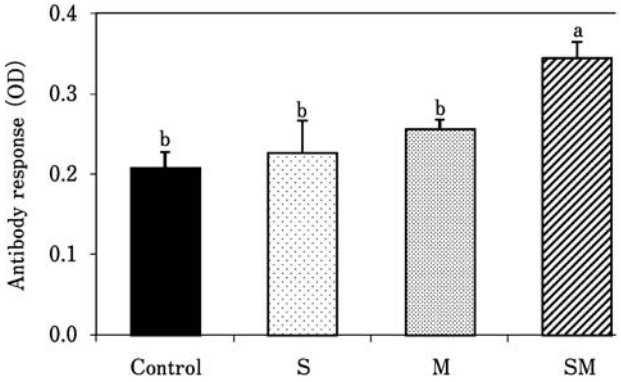

Fig. 3. **The effect of dietary plants on EtMIC2 serum antibody responses of birds infected with *E. acervulina*.** One-day-old broiler chickens were fed a standard diet alone (Control) or a standard diet supplemented with *C. annuum* and *C. frutescens* plus *L. edodes* (S), *C. longa* (M), or *C. annuum* and *C. frutescens, L. edodes,* and *C. longa* (SM). At 14 days post-hatch, chickens were infected with 2.0×10^4 sporulated oocysts of *E. acervulina.* Serum samples were obtained at day 10 post-infection (4 birds/group) and tested by ELISA against a recombinant antigen EtMIC2. Each bar represents the mean±SEM values. Bars not sharing the same letters are significantly different ($P<0.05$) according to the Duncan's multiple range test.

Local Cytokine Production

As shown in Figure 4A, the level of transcripts encoding the pro-inflammatory cytokine IL-1β in the intestinal duodenum was significantly increased in uninfected chickens fed the SM diet at day 14 post-hatch compared with the control group ($P<0.05$). Furthermore, the levels of transcripts encoding IL-6, IL-15, and IFN-γ were also increased in SM group compared with controls (Figures 4B, 4C, 4D). Of the 4 cytokines examined, IL-15 was significantly increased in all 3 groups with supplemented diets compared to the control group (Figure 4C).

Discussion

SM diet supplemented with *C. longa* and *C. annuum* and *-frutescens* in combination with *L. edodes* provided significant body weight gains following infection with *E. acervulina* when compared to standard diet or diets containing *C. annuum* and *C. frutescens/L. edodes.* Furthermore, only the combination of *Curcuma/Capsicum/Lentinus* (SM diet) was efficient in reducing fecal oocyst shedding and increasing the titers of serum antibodies reactive with EtMIC2, an apical complex protein which plays an important role in host cell invasion of *Eimeria* parasites (Tomley *et al.,* 1996; Lillehoj and Lillehoj, 2000). Taken together, these results provide clear evidence for an

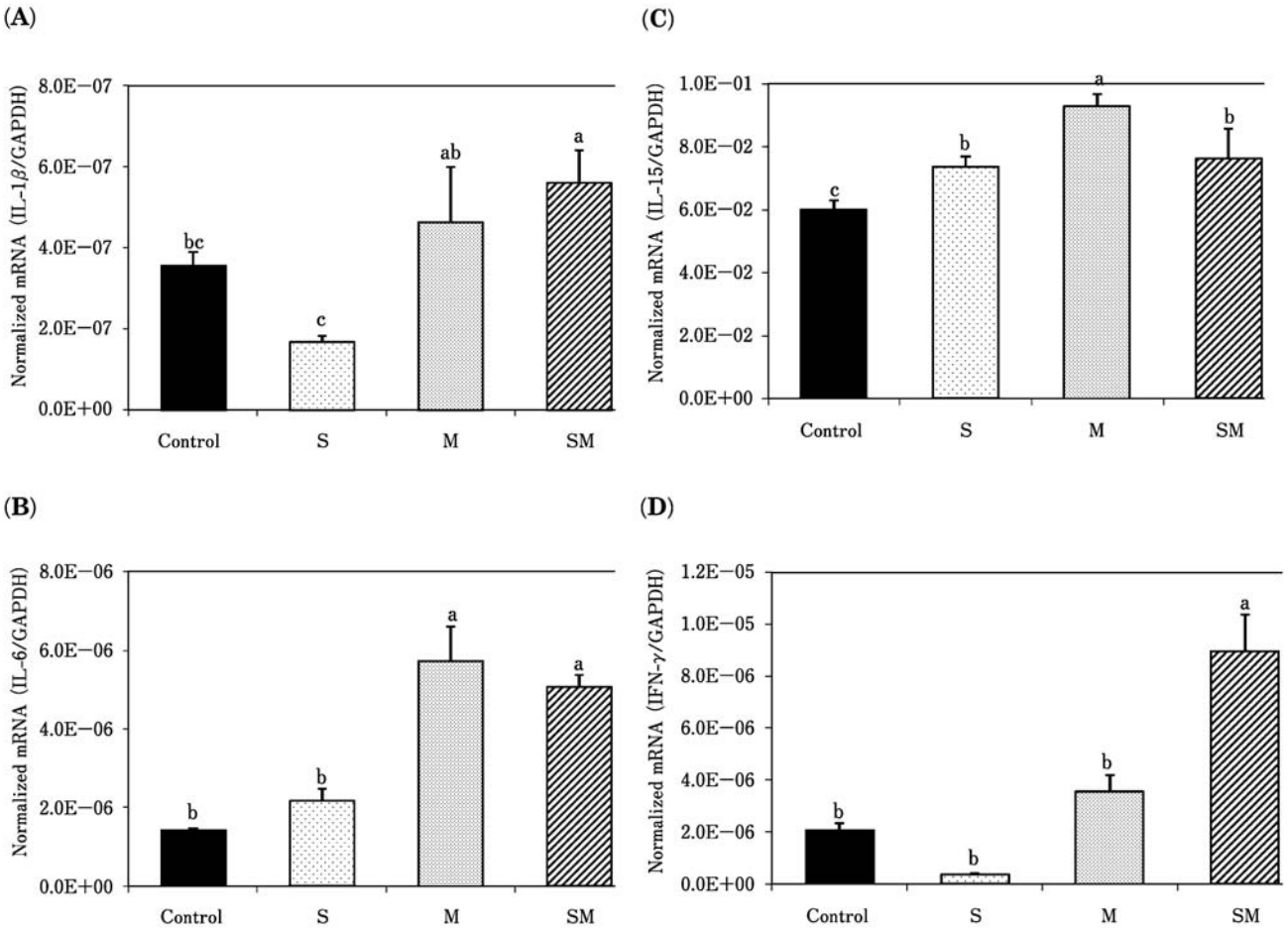

Fig. 4. **The effect of dietary plants on pro-inflammatory cytokine transcript levels of birds uninfected with *E. acervulina*.** One-day-old broiler chickens were fed a standard diet alone (Control) or a standard diet supplemented with *C. annuum* and *C. frutescens* plus *L. edodes* (S), *C. longa* (M), or *C. annuum* and *C. frutescens, L. edodes,* and *C. longa* (SM). At 14 days post-hatch, the intestinal duodenum was removed and the levels of transcripts for IL-1β (A), IL-6 (B), IL-15 (C), and IFN-γ (D) were quantified by real time RT-PCR. Each bar represents the mean$\pm$SEM. Bars not sharing the same letters are significantly different ($P<0.05$) according to the Duncan's multiple range test.

effect of the different phytonutrient combinations in promoting local protective immunity against experimental avian coccidiosis. In addition, only the SM diet induced all four of the cytokines in the gut, further supporting the effective action of all 3 plants in stimulating local immunity. Alternatively stated, while all seven parameters of protective immunity to coccidiosis were augmented using the *Curcuma/Capsicum/Lentinus*-supplemented diet, *C. longa*-supplemented M diet also enhanced weight gain, IL-6, and IL-15 expression. Because IL-15 plays an important role in the development, survival, and function of NK cells (Waldmann and Tagaya, 1999) and acts as a memory-facilitating factor for helper T cells (Zhang *et al.,* 1998; Kanegane and Tosato, 1996), future studies on the effect of feeding these plants in promoting prolonged vaccinal immune response will be valuable.

Historically, the severity of experimental *Eimeria* infection in chickens has been assessed by loss of body weight gain, excretion of fecal oocysts, and the presence of intestinal lesions (Idris *et al.,* 1997). These disease parameters reflect host immunity status in avian coccidiosis (Lillehoj *et al.,* 2007c). The challenge dose of *E. acervulina* that was used in this investigation is likely to be considerably higher than exposure levels that commercial flocks encounter (Wallach *et al.,* 1995). Therefore, lower doses of the turmeric/hot pepper/shiitake mixture supplemented in standard poultry feed may still provide effective protection against coccidiosis in poultry raised under normal field conditions.

Given that markers of both humoral immunity (*Eimeria*-specific antibodies) and cell-mediated immunity (pro-inflammatory cytokines) were enhanced by the plant-supplemented diet, it will be important to investigate the cellular and molecular mechanisms responsible for immunoenhancing effects of these plants. Because cell-mediated immunity has been shown to play a major role in

protection against avian coccidiosis (Lillehoj and Ruff, 1987; Lillehoj and Trout, 1996; Lee *et al.,* 2007b, c), it is possible that *Curcuma, Capsicum,* and *Lentinus,* when combined together, show increased effectiveness on stimulating local cell-mediated immunity protective against coccidiosis. In this study, *Curcuma/Capsicum/Lentinus* (SM diet) also enhanced serum antibody levels against a microneme protein 2 from *E. tenella* (EtMIC2). EtMIC2 has a putative function in parasite adhesion to the host cell and plays an important role in inhibiting sporozoite invasion of host cells (Sasai *et al.,* 2008). *Curcuma/Capsicum/Lentinus*-treated birds showed higher antibody response to EtMIC2 protein and shed less oocysts. Therefore, increasing the level of serum antibodies which are directed against parasite antigens of survival importance will likely enhance local protection against coccidiosis.

Host immune response to *Eimeria* is accompanied by series of cell-mediated immune responses and several cytokines including IFN-γ, IL-1β, IL-6, and IL-15 which are involved in local inflammatory responses (Lillehoj *et al.,* 2001, 2007c; Lee *et al.,* 2008c; Hong *et al.,* 2006a, b). IFN-γ is a common marker of cellular immunity and high levels are associated with protective immune responses to coccidiosis (Min *et al.,* 2003; Lillehoj *et al.,* 2004; Lee *et al.,* 2008a). Administration of recombinant IFN-γ to chickens increased host protection against coccidiosis, significantly reduced the intracellular development of *Eimeria* parasites (Lillehoj and Trout, 1996), and showed adjuvant effect when given in a DNA vaccine (Min *et al.,* 2001). IL-1β is a pro-inflammatory cytokine produced by macrophages, monocytes and dendritic cells, and is an important mediator of innate immunity. In mammals, IL-1β increases the expression of adhesion factors on endothelial cells to enable the transmigration of leukocytes to the sites of infection. IL-1β, when given simultaneoulsy with a DNA vaccine, exerted an adjuvant effect by reducing fecal oocyst shedding following an oral infection with *Eimeria* (Min *et al.,* 2001). IL-6 is produced by T-cells and macrophages and acts as both a pro-inflammatory and an anti-inflammatory cytokine whereas IL-15, primarily secreted by mononuclear phagocytes, enhances the activation of memory T cells (Waldmann and Tagaya, 1999; Kanegane and Tosato, 1996). In chickens, IL-15 promoted the survival of T-lymphocytes and NK cells (Lillehoj *et al.,* 2001; Choi and Lillehoj, 2002) and enhanced protective immunity to coccidiosis when co-administered with a DNA vaccine (Lillehoj *et al.,* 2001; Min *et al.,* 2001). Enhanced production of these cytokines in birds which were continously fed with the *Curcuma/Capsicum/Lentinus*-supplemented diet provides a new opportunity to utilize these dietary phytonutrients to increase local innate immunity and to reduce economic losses due to coccidiosis.

In conclusion, our results provide the first demonstration that a combination of *Curcuma, Capsicum, and Lentinus* effectively enhances the disease resistance of birds to *E. acervulina* infection. Although further studies are necessary to better understand the underlying immune mechanisms which are responsible for the dietary immune enhancement against avian coccidiosis using these plants, this study provides clear immunological evidence for their role in stimulating humoral and cell-mediated immunity in poultry. Furthermore, for a complex intestinal parasitic infection such as coccidiosis whose treatment has traditionally been relied upon prophylactic medication, dietary immune enhancement using food plants provides a safe alternative control method to reduce economic losses due to coccidiosis in poultry.

Acknowledgments

This project was partially supported by a formal Trust agreement established between ARS-USDA and Pancosma S.A. The authors thank Margie Nichols for her significant contribution to this research.

References

Banfield MJ, Kwakkel RP and Forbes JM. Effects of wheat structure and viscosity on coccidiosis in broiler chickens. Animal Feed Science and Technology, 98: 37–48. 2002.

Choi KD and Lillehoj HS. Role of chicken IL-2 on $\gamma\delta$ T-cells and Eimeria acervulina-induced changes in intestinal IL-2 mRNA expression and $\gamma\delta$ T-cells. Veterinary Immunology and Immunopathology, 73: 309–321. 2000.

Conforti F, Statti GA and Menichini F. Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. acuminatum L.) in relation to maturity stage. Food Chemistry, 102: 1096–1104. 2007.

Hong YH, Lillehoj HS, Lillehoj EP and Lee SH. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following *Eimeria maxima* infection of chickens. Veterinary Immunology and Immunopathology, 114: 259–272. 2006a.

Hong YH, Lillehoj HS, Lee SH, Dalloul RA and Lillehoj EP. Analysis of chicken cytokine and chemokine gene expression following *Eimeria acervulina* and *Eimeria tenella* infections. Veterinary Immunology and Immunopathology, 114: 209–223. 2006b.

Idris AB, Bounous DI, Goodwin MA, Brown J and Krushinskie EA. Lack of correlation between microscopic lesion scores and gross lesion scores in commercially grown broilers examined for small intestinal *Eimeria* spp. coccidiosis. Avian Diseases, 41: 388–391. 1997.

Kanegane H and Tosato G. Activation of naive and memory T cells by interleukin-15. Blood, 88: 230–235. 1996.

Lee SH, Park JB, Park HJ, Park YJ and Sin JI. Biological properties of different types and parts of the dandelions: comparisons of anti-oxidative, immune cell proliferative and tumor cell growth inhibitory activities. Journal of Food Science and Nutrition, 10: 172–178. 2005.

Lee SH, Lillehoj HS, Park DW, Hong YH, Cho SM, Chun HK and Park HJ. Immunomodulatory effects of dietary safflower leaf in chickens. Korean Journal of Community Living Science, 18: 715–724. 2007a.

Lee SH, Lillehoj HS, Dalloul RA, Park DW, Hong YH and Lin JJ. Influence of *Pediococcus*-based probiotic on coccidiosis in broiler chickens. Poultry Science, 86: 63–66. 2007b.

Lee SH, Lillehoj HS, Park DW, Hong YH and Lin JJ. Effects of *Pediococcus* and *Saccharomyces*-based probiotic (MitoMax®)

on coccidiosis broiler chickens. Comparative Immunology, Microbiology and Infectious Diseases, 30: 261–268. 2007c.

Lee SH, Lillehoj HS, Chun HK, Tuo W, Park HJ, Cho SM, Lee YM. and Lillehoj EP. *In vitro* treatment of chicken peripheral blood lymphocytes, macrophages, and tumor cells with extracts of Korean medicinal plants. Nutrition Research, 27: 362–366. 2007d.

Lee SH, Lillehoj HS, Cho SM, Park DW, Hong YH, Chun HK and Park HJ. Immunomodulatory properties of dietary plum on coccidiosis. Comparative Immunology, Microbiology and Infectious Diseases, 31: 389–402. 2008a.

Lee SH, Lillehoj HS, Chun HK, Park HJ, Cho SM and Lillehoj EP. *In vitro* effects of methanol extracts of Korean medicinal fruits (Persimmon, Raspberry, Tomato) on chicken lymphocytes, macrophages, and tumor cells. Journal of Poultry Science, 46: 149–154. 2008b.

Lee SH, Lillehoj HS, Cho SM, Park DW, Hong YH, Lillehoj EP, Heckert RA, Park HJ and Chun HK. Protective effects of dietary safflower (*Carthamus tinctorius*) on experimental coccidiosis. Journal of Poultry Science, 46: 155–162. 2008c.

Lee SH, Lillehoj HS, Park DW, Jang SI, Morales A, García D, Lucio E, Larios R, Victoria G, Marrufo D and Lillehoj EP. Effect of hyperimmune egg yolk IgY against *Eimeria tenella* and *Eimeria maxima* in broiler chickens. Poultry Science, 88: 562–566. 2008d.

Lillehoj HS and Ruff MD. Comparison of disease susceptibility and subclass-specific antibody response in SC and FP chickens experimentally inoculated with *Eimeria tenella, E. acervulina,* or *E. maxima.* Avian Diseases, 31: 112–119. 1987.

Lillehoj HS and Trout JM. Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. Clinical Microbiology Reviews, 9: 349–360. 1996.

Lillehoj HS. Role of T lymphocytes and cytokines in coccidiosis. International Journal for Parasitology, 28: 1071–1081, 1998.

Lillehoj HS and Lillehoj EP. Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Diseases, 44: 408–425. 2000.

Lillehoj HS, Min W, Choi KD, Babu US, Burnside J, Miyamoto T, Rosenthal BM and Lillehoj EP. Molecular, cellular, and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-2. Veterinary Immunology and Immunopathology, 82: 229–244. 2001.

Lillehoj HS, Min W and Dalloul RA. Recent progress on the cytokine regulation of intestinal immune response to *Eimeria.* Poultry Science, 83: 611–623. 2004.

Lillehoj HS and Lee SH. Probiotics as an alternative control strategy against avian coccidiosis. Feedinfo News Service Scientific Reviews. http: //www.feedinfo.com. 2007a.

Lillehoj HS and Lee SH. Dietary modulation of intestinal innate immunity using plant-derived phytochemicals. Feedinfo News Service Scientific Reviews. http: //www.feedinfo.com. 2007b.

Lillehoj HS, Kim CH, Keeler Jr CL and Zhang S. Immunogenomic approaches to study host immunity to enteric pathogens. Poultry Science, 86: 1491–1500. 2007c.

Mannangatti K and Narayanasamy M. Antifungal protein from a medicinal plant, *Curcuma caesia* Roxb. Journal of Biotechnology, 136: S90. 2008.

Min W, Lillehoj HS, Burnside J, Weining KC, Staeheli P and Zhu JJ. Adjuvant effects of IL-1β, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TGF-β4 and lymphotactin on DNA vaccination against *Eimeria acervulina.* Vaccine, 20: 267–274. 2001.

Min W, Lillehoj HS, Kim S, Zhu JJ, Beard H, Alkharouf N and Matthews BF. Profiling local gene expression changes associated with *Eimeria maxima* and *Eimeria acervulina* using cDNA microarray. Applied Microbiology and Biotechnology, 62: 392–399. 2003.

Muller PY, Janovjak H, Miserez AR and Dobbie Z. Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques, 32: 1372–1379. 2002.

Naidoo V, McGaw LJ, Bisschop SPR, Duncan N and Eloff JN. The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Veterinary Parasitology, 153: 214–219. 2008.

Oboh G, Puntel RL and Rocha JBT. Hot pepper (*Capsicum annuum,* Tepin and *Capsicum chinese,* Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain—*in vitro.* Food Chemistry, 102: 178–185. 2007.

Park JM, Lee SH, Kim JO, Park HJ, Park JB and Sin JI. *In vitro* and *in vivo* effects of extracts of *Lentinus edodes* on tumor growth in a human papilloma virus 16 oncogenes-transformed animal tumor model—Apoptosis-mediated tumor cell growth inhibition. Korean Journal of Food Science and Technology, 36: 141–146. 2004.

Policegoudra RS, Abiraj K, Gowda DC and Aradhya SM. Isolation and characterization of antioxidant and antibacterial compound from mango ginger (*Curcuma amada* Roxb.) rhizome. Journal of Chromatography B, 852: 40–48. 2007.

Sasai K, Fetterer RH, Lillehoj H, Matusra S, Constantinoiu C, Matsubayashi M, Tani H and Baba E. Characterization of monoclonal antibodies that recognize the *Eimeria tenella* microneme protein MIC2. Journal of Parasitology, 94: 1432–1434. 2008.

Sodsai A, Piyachaturawat P, Sophasan S, Suksamrarn A and Vongsakul M. Suppression by *Curcuma comosa* Roxb. of pro-inflammatory cytokine secretion in phorbol-12-myristate-13-acetate stimulated human mononuclear cells. International Immunopharmacology, 7: 524–531. 2007.

Spices board. Spice India. Niseema Prnters & Publishers, Cochin. 21: 1–52. 2008.

Tomley FM, Bumstead JM, Billington KJ and Dunn PP. Molecular cloning and characterization of a novel acidic microneme protein (Etmic-2) from the apicomplexan protozoan parasite, *Eimeria tenella.* Molecular and Biochemical Parasitology, 79: 195–206. 1996.

Waldmann TA and Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. Annual Review of Immunology, 17: 19–49. 1999.

Wallach M, Smith NC, Petracca M, Miller CM, Eckert J and Braun R. *Eimeria maxima* gametocyte antigens: potential use in a subunit maternal vaccine against coccidiosis in chickens. Vaccine, 13: 347–354. 1995.

Zhang X, Sun S, Hwang I, Tough DF and Sprent J. Potent and selective stimulation of memory-phenotype $CD8^+$ T cells in vivo by IL-15. Immunity, 8: 591–599. 1998.